

REMARKS

Applicant and his counsel thank the Examiner for the courtesy of an interview on May 12, 2003, in which the § 102(b) rejection over Drebin, the § 112, first and second paragraph rejections of all of the pending claims, and potential obviating amendments were discussed. Several limitations were discussed and are incorporated into the amended claims presented herein.

Claims 33, 34, 36, 37, 43-50, 59-65 and 71-78 were examined. Claims 33, 43, 59, 63, and 71 are amended. Claims 87-89 are new. Claims 87 is dependent and recites specific enzymes that are supported by the specification, for example, at page 13, lines 21-30 ('281 patent, col. 6, lines 45-54) and at page 18, lines 11-13 ('281 patent, col. 8, lines 24-26). Claim 88 is dependent and is directed to methods wherein the phenotypic response that is evoked is measured during performance of claimed assay method. Support is found in the Specification, for example, at page 34, line 28 - page 35, line 13 (U.S. Patent No. 4,980,281, the "'281 patent," col. 16, lines 8-29) and Fig. 4 as described therein (prior to treatment with activator, the test and control cell monolayers displayed the characteristic fusiform morphology of the parental cell line - the phenotypic response was measured following addition of TPA (a chemical agent that is a phorbol ester tumor promoter which is known to directly activate the cellular functioning of PKC) and a 24 hour incubation period) and at page 38, line 26 - page 39, line 6 ('281 patent, col. 18, lines 3-17) and Table 3 (individual cells were seeded in soft agar, and subsequently treated with various inhibitors and cloning efficiency measured after a 30 day incubation period). Claim 89 is dependent and is directed to methods wherein the phenotypic response the is evoked is modulation of phosphorylation of an intracellular protein substrate of the enzyme. Support is found in the Specification, for example, at page 14, lines 11-16 ('281 patent, col. 6, line 63 - col. 7, line 1).

Rejection Under 35 U.S.C. § 102(b)

Claims 33-34, 36, 43-44, 46-47, 49, 63-64, 71-72, 74-75, and 77 stand rejected under 35 U.S.C. § 102(b) as anticipated by Drebin et al. ("Drebin").

For reasons already of record, and as discussed during the interview, Applicant respectfully maintains the position that Drebin does not anticipate the rejected claims at least because "binding and internalization" is not inhibition or activation of an enzyme – as that

term is used in the specification. Such mechanisms of “down modulation” or “down regulation,” whereby the enzyme (protein of interest, or “POI”) is internalized and destroyed by the cell, lie outside the meaning of the terms “activator” and “inhibitor” as disclosed by the specification. It also remains Applicant’s position that Drebin’s antibodies are not chemical agents – as that term is used in the specification. The terms “chemical inhibitor” and “chemical activator” (specification, page 8, lines 22-25; ‘281 patent, col. 4, lines 41-44) appropriately refer to agents other than antibodies. One of ordinary skill in the art, at the time the application was filed, would not have included antibodies within the meaning of the term chemical agents. Further, it remains Applicant’s position that Drebin’s cells do not display a phenotypic characteristic that is responsive to inhibitors or activators of the enzyme. In causing the removal of p185 from the cell surface, Drebin does no more than convert a test cell to a control cell. One of ordinary skill in the art, referring to the work of Drebin as well as to work described in other references cited therein (see Drebin, page 696, col. 2, lines 20-25) would know that antibodies directed against cell surface receptors are known to cause down regulation of the corresponding receptor, and therefore that such an approach could preclude the ability to practice Applicant’s invention.

However, Applicant has amended the claims to clarify that the instant claims are limited to the embodiment in which the level of the POI is maintained such that the cell is capable of exhibiting the phenotypic characteristic upon removal of the chemical agent. This means that the cell remains capable of responding to activators or inhibitors of the POI, even after removal of an active test substance. Such would not be the result where the level of the POI in the cell was substantially diminished as a consequence of treatment with an active test substance.

Modulation of the *responsive change in a phenotypic characteristic* (i.e., a change in the *phenotypic response*) which is the claimed test, will be due primarily to a change in the activity of the enzyme in the cell, rather than a change in the amount of the enzyme in the cell. In other words, it is a change in the activity of the enzyme that is generally observed in the presence of activators or inhibitors according to the methods of the invention, rather than merely the level of the enzyme. Indeed, it is the substantially increased potential activity of the enzyme in the test cell that makes the cell become highly sensitive and responsive to activators or inhibitors of the enzyme, *i.e.*, chemical agents which interact with (bind to) the

enzyme in order to exert their inhibitory or activating effects on the cellular functioning of the target protein. For convenience, passages of the specification are reproduced:

"The cell lines which resulted from the application of this method are highly sensitive and responsive both to agents which activate PKC as well as to those which inhibit PKC."

Specification, page 23, lines 3-6; '281 patent, col. 10, lines 26-29.

Thus, this work establishes, for the first time, the fact that stable overproduction of a protein in mammalian cells can result in a novel cellular phenotype(s) (in this case anchorage independence) which can be directly modulated by *chemical agents which interact with the protein*.

Specification, page 39, lines 21-26; '281 patent, col. 18, lines 30-36 (emphasis added).

As taught in detail in the specification, under appropriate culture conditions the skilled investigator provides a cell that, in the words of the claims, is able to exhibit the phenotypic response when the protein is activated or inhibited *relative to* a control cell that exhibits the response to a lesser degree or not at all. (See, e.g., specification, page 12, line 30 - page 13, line 7; '281 patent, col. 6, lines 21-33, specification, page 10, lines 7-11; '281 patent, col. 5, lines 23-27).

Most importantly, the significance of providing an appropriate test cell capable of maintaining a functional level of the POI in the presence of the chemical agent is discussed in detail in the specification, for example, at page 35, line 15 through page 36, line 5 (Example 1; '281 patent, col. 16, lines 45-55), wherein it is explained that normal cells, upon activation of the enzyme PKC by the chemical agent TPA, "down-regulate" or "down-modulate" PKC, and thus become refractory to further stimulation. Such down regulation of PKC upon stimulation was known in the art. (See, e.g., Boreiko et al., 1980, Cancer Res. 40, 4709-16, cited in the original specification, and of record in the instant application.) In contrast, Applicant's test cells that maintain high levels of PKC (e.g., constitutive production) are demonstrated to respond to TPA in a repetitive manner and *do not* become refractory. Stable expression of the POI leads to a measurable / observable change in morphology of the cells in response to treatment with the PKC activator TPA that continues to be sensitive to subsequent treatments with TPA. Applicant's test cell remains responsive following treatment with an active chemical agent. In contrast Drebin teaches that p185 is sufficiently

down-modulated following exposure to the antibody that the cells lose their phenotype altogether (see, *e.g.*, Drebin, page 697, Fig. 3B, page 699, Fig. 6, and discussion therein).

Applicant points out, however, that the level of the enzyme need not remain exactly the same in the presence of the chemical test agent. It is sufficient that the enzyme be maintained at a high level in the test cell relative to the control cell such that modulation of the responsive change in the phenotypic characteristic by inhibitors and activators can be observed or measured.

We also note that Drebin has not been applied against Claims 37, 45, 50, 59-62, 65, 73, 76 and 78. In view of the amendments to the claims, we submit that the rejection over Drebin of Claims 33-34, 36, 43-44, 46-47, 49, 63-64, 71-72, 74-75, and 77 should be withdrawn.

Rejections Under 35 U.S.C. § 112, first and second paragraph

The Examiner has maintained three separate rejections of Claims 33-34, 36-37, 43-50, 59-65, and 71-78 under 35 U.S.C. § 112, first and second paragraphs for written description and enablement.

The Examiner rejected these claims under 35 U.S.C. § 112, first paragraph for lack of enablement, because the preamble of the rejected claims requires, *a priori*, knowledge concerning the direct interaction between the test chemical agent and the target enzyme. The preamble has been amended to clarify that no such *a priori* knowledge is necessary.

Applicant concurs with the Examiner that a false positive result – namely where modulation of a responsive change in a phenotypic characteristic of the cell which does not arise from (direct) inhibition or activation of the enzyme (*i.e.*, where the test substance interacts with (binds to) some *other* protein in the cell) – is theoretically possible. False positive results are possible with all assay systems. Nevertheless, Applicant continues to maintain that practice of his test system unexpectedly results in a high probability of instances in which a chemical agent produces a responsive change in the phenotypic characteristic due to inhibiting or activating the enzyme – that is, through the direct binding of the chemical agent to the POI. (See, *e.g.*, specification, page 5, lines 14-19, '281 patent, col. 3, lines 5-10; specification, page 12, lines 22-28, '281 patent, lines 14-20; specification, page 23, lines 3-6, '281 patent, col. 10, lines 26-29, reproduced above; and specification, page 39, lines 21-26, '281 patent, col. 18, lines 30-36, reproduced above).

Furthermore, Applicant wishes to emphasize that he has used his system to identify, for the first time, the ability of tamoxifen to inhibit the cellular functioning of the $\beta 1$ isoform of PKC (PKC $\beta 1$) (specification, page 38, line 26 - page 39, line 26 and Table 3; '281 patent, col. 18, lines 30-36, Table 3). Prior to Applicant's demonstration of this result using his patented method, however, it had not been shown that tamoxifen could bind to (interact with) PKC. Binding of tamoxifen to PKC was subsequently disclosed in a manuscript co-authored by Applicant (O'Brian et al., 1988, *Cancer Res.* 48:3626-29; Exhibit A).

Kinetic studies provide evidence that the inhibition of PKC by tamoxifen involves *nonspecific* interactions between the antiestrogen and the lipid cofactor (13-15). However, the possibility that tamoxifen also has direct interactions with PKC has not been addressed prior to this report. Here we show that PKC *binds to* an immobilized analogue of tamoxifen *directly and specifically*.

O'Brian et al. (Exhibit A) at p. 3626, paragraph spanning cols. 1 and 2 (emphasis added).

A method need not be infallible to be enabled. A claim is not invalid simply if it encompasses some inoperative embodiments. *Atlas Powder Co. v. E. I. duPont de Nemours & Co.*, 750 F.2d 1569, 1576, 224 U.S.P.Q. 409, 414 (Fed. Cir. 1984). See, also, *In re Geerdes*, 491 F.2d 1260, 1265, 180 U.S.P.Q. 789, 793 (CCPA 1974) ("... it is possible to argue that process claims encompass inoperative embodiments on the premise of unrealistic or vague assumptions, but that is not a valid basis for rejection.")

Nevertheless, Applicant appreciates the Examiner's position that false positive results are a possibility. Therefore, in the interest of facilitating allowance Applicant has amended the claims to provide that a conventional *in vitro* binding assay may also be performed to establish, confirm, determine, etc. that the chemical agent does indeed interact with (bind to) the enzyme as well as inhibit or activate the cellular functioning of the enzyme. Naturally, such assays can be performed either before or after steps (a) – (d) (independent Claims 33, 59, and 63) or steps (a) – (c) (independent Claims 43 and 71). For example, binding of PDBU to PKC in cell lines expressing the $\beta 1$ isoform of PKC is exemplified at page 31, line 5 - page 32, line 15 (specification, page 31, line 5 - page 32, line 15; '281 patent, col. 14, lines 19-63), and Horowitz, et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:2315-2319 (referred to in the cited portion of the Specification).

Applicant submits that the same amendments resolve the rejection under § 112, first paragraph for lack of description.

Claims 33-34, 36-37, 43-50, 59-65, and 71-78 are rejected under 35 U.S.C. § 112, second paragraph as indefinite with respect to the nature of control and test cell lines and genetic vectors. Applicant maintains its position, for the reasons expressed in Applicant's previous response, that the control cell lines are sufficiently definite to those with ordinary skill in the art. However, Applicant has also amended the claims in an effort to reach agreement on this issue as well. As amended, the claims recite that the second cell line (or control cell) is alike to the first cell line (or test cell), except for the level of the target enzyme (POI) and the responsive change in a phenotypic characteristic (phenotypic response) that is evoked. Support for the amendment is found in the specification, for example, at page 8, lines 25-29 ('281 patent, col. 4, lines 44-48). The relationship of the first and second cell lines is discussed throughout the specification. In preferred embodiments, the first cell line is constructed to be the same as the second cell line, except for the expression vector that is introduced to create the first cell line. In one preferred embodiment, the first cell line is a host cell line into which a vector expressing the enzyme has been inserted, whereas the second cell line is the host cell line itself. In another preferred embodiment, the first cell line is a host cell line into which a first vector expressing the enzyme has been inserted, whereas the second cell line is the host cell line into which a second vector that does not express the enzyme, but is otherwise identical to the first vector, has been inserted. (See, *e.g.*, specification, page 9, line 32 - page 10, line 14, '281 patent, col. 5, lines 13-30; specification, page 19, lines 18-24, '281 patent, col. 8, lines 46-52). However, Applicant points out that the method of the invention is functional where there are insubstantial differences between the cell lines. (See, *e.g.*, specification, page 8, lines 25-29, '281 patent, col. 4, lines 44-48). In addition, by reciting that the level of the enzyme is maintained at a level such that the cell is capable of exhibiting said phenotypic response following removal of a direct activator or inhibitor of the enzyme, the amended claims more clearly set forth that the first (test) and second (control) cell lines of the claims are differentiated by their expression of the enzyme at different levels in the presence of a test agent.

It is believed that this amendment is fully responsive to the Examiner's rejection and allowance of the claims is respectfully requested.

Respectfully submitted,

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Version of amended claims to show changes made

33. (amended) A method of determining whether a chemical agent [that directly interacts with an enzyme] is a direct [an] inhibitor or activator of [that] an enzyme in a cell whose production by [a] that cell evokes a responsive change in a phenotypic characteristic of the cell, other than the level of said enzyme in said cell per se, which comprises:

(a) providing a first mammalian cell line which produces said enzyme and exhibits said phenotypic response to the enzyme and wherein the level of the enzyme in the cell is maintained such that the cell is capable of exhibiting the phenotypic response following removal of a direct activator or inhibitor of the enzyme;

(b) providing a second mammalian cell line which is alike to the first mammalian cell line, but which produces the enzyme at a lower level than the first cell line, or does not produce the enzyme at all, and which exhibits said phenotypic response to the enzyme to a lesser degree or not at all;

(c) incubating the chemical agent with the first and second cell lines; [and]

(d) comparing the phenotypic response of the first cell line to the chemical agent with the phenotypic response of the second cell line to the chemical agent; and

(e) determining through the use of a binding assay that the chemical agent binds to the enzyme.

43. (amended) A method of determining whether a chemical agent [that directly interacts with an enzyme] is [an] a direct inhibitor or activator of [that] an enzyme in a cell which comprises:

(a) providing a mammalian test cell which overproduces a selected enzyme relative to a mammalian control cell which is alike to the test cell, but which produces said enzyme at a lower level or essentially does not produce the enzyme, and wherein production of said enzyme in said test cell evokes a responsive change in a phenotypic characteristic of said test cell, other than the level of said enzyme in said test cell per se, which is comparatively

greater than in said control cell, and wherein the level of the enzyme in the cell is maintained such that the cell is capable of exhibiting the phenotypic response following removal of a direct activator or inhibitor of the enzyme;

(b) treating said test cell containing the overproduced selected enzyme with said chemical agent; [and]

(c) examining the treated test cell to determine whether it exhibits a change in said phenotypic characteristic in response to said chemical agent; and

(d) determining through the use of a binding assay that the chemical agent binds to the enzyme.

59. (amended) A method of determining whether a chemical agent [that directly interacts with an enzyme] is a direct [an] inhibitor or activator of [that] an enzyme in a cell whose production by [a] that cell evokes a responsive change in a phenotypic characteristic of the cell, other than the level of the enzyme in said cell per se, which comprises:

(a) providing a first mammalian cell line which produces the enzyme and exhibits the phenotypic response to the enzyme and wherein the level of the enzyme in the cell is maintained such that the cell is capable of exhibiting the phenotypic response following removal of a direct activator or inhibitor of the enzyme, said first cell line obtained by introducing a gene encoding the enzyme into a first host cell by means of a first genetic vector into which said gene has been inserted, said gene being under the control of a promoter functional in said host cell, whereby said gene is expressed;

(b) providing a second mammalian cell line which is alike to the first mammalian cell line, but which produces the enzyme at a lower level than said first cell line, or does not produce the enzyme at all, and which exhibits the phenotypic response to the enzyme to a lesser degree or not at all, said second cell line obtained by introducing into a [similar] second host cell which is alike to the first host cell, a second genetic vector essentially identical to said first genetic vector except that it does not bear said gene insert;

- (c) incubating the chemical agent with said first and second cell lines; [and]
- (d) comparing the phenotypic response of said first cell line to the chemical agent with the phenotypic response of said second cell line to the chemical agent; and
- (e) determining through the use of a binding assay that the chemical agent binds to the enzyme.

63. (amended) A method of determining whether a chemical agent [that directly interacts with an enzyme] is a direct [an] inhibitor or activator of [that] an enzyme whose production by a cell evokes a responsive change in a phenotypic characteristic, other than the level of the enzyme in the cell per se, which comprises:

- (a) providing a first mammalian cell line which produces the enzyme and exhibits the phenotypic response to the enzyme and wherein the level of the enzyme in the cell is maintained such that the cell is capable of exhibiting the phenotypic response following removal of a direct activator or inhibitor of the enzyme, said phenotypic response being a graded cellular response;
- (b) providing a second mammalian cell line which is alike to the first mammalian cell line, but which produces the enzyme at a lower level than said first cell line, or does not produce the enzyme at all, and which exhibits the phenotypic response to the enzyme to a lesser degree or not at all;
- (c) incubating the chemical agent with said first and second cell lines; [and]
- (d) comparing the graded cellular response of said first cell line to the chemical agent with the phenotypic response of said second cell line to the chemical agent; and
- (e) determining through the use of a binding assay that the chemical agent binds to the enzyme.

71. (amended) A method of determining whether a chemical agent [that directly interacts with an enzyme] is a direct [an] inhibitor or activator of [the] an enzyme which comprises:

(a) providing a mammalian test cell which overproduces the selected enzyme relative to a mammalian control cell which is alike to the test cell, but which produces the enzyme at a lower level or essentially does not produce the enzyme, and wherein production of the enzyme in said test cell evokes a responsive change in a phenotypic characteristic of said test cell, other than the level of the enzyme in said test cell per se, said responsive change being a graded cellular response, which is comparatively greater than in said control cell, and wherein the level of the enzyme in the cell is maintained such that the cell is capable of exhibiting the phenotypic response following removal of a direct activator or inhibitor of the enzyme;

(b) treating said test cell containing the overproduced selected enzyme with the chemical agent; [and]

(c) examining said treated test cell to determine whether it exhibits a change in said graded cellular response to the chemical agent; and

(d) determining through the use of a binding assay that the chemical agent binds to the enzyme.